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Fate of Naturally Occurring *Escherichia coli* O157:H7 and Other Zoonotic Pathogens during Minimally Managed Bovine Feedlot Manure Composting Processes†

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ABSTRACT

Reducing *Escherichia coli* O157:H7 in livestock manures before application to cropland is critical for reducing the risk of foodborne illness associated with produce. Our objective was to determine the fate of naturally occurring *E. coli* O157:H7 and other pathogens during minimally managed on-farm bovine manure composting processes. Feedlot pen samples were screened to identify *E. coli* O157:H7–positive manure. Using this manure, four piles of each of three different composting formats were constructed in each of two replicate trials. Composting formats were (i) turned piles of manure plus hay and straw, (ii) static stockpiles of manure, and (iii) static piles of covered manure plus hay and straw. Temperatures in the tops, toes, and centers of the conical piles (ca. 6.0 m^3 each) were monitored. Compost piles that were turned every 2 weeks achieved higher temperatures for longer periods in the tops and centers than did piles that were left static. *E. coli* O157:H7 was not recovered from top samples of turned piles of manure plus hay and straw at day 28 and beyond, but top samples from static piles were positive for the pathogen up to day 42 (static manure stockpiles) and day 56 (static covered piles of manure plus hay and straw). *Salmonella, Campylobacter* spp., and *Listeria monocytogenes* were not found in top or toe samples at the end of the composting period, but *E. coli* O157:H7 and *Listeria* spp. were recovered from toe samples at day 84. Our findings indicate that some minimally managed composting processes can reduce *E. coli* O157:H7 and other pathogens in bovine manure but may be affected by season and/or initial levels of indigenous thermophilic bacteria. Our results also highlight the importance of adequate C:N formulation of initial mixtures for the production of high temperatures and rapid composting, and the need for periodic turning of the piles to increase the likelihood that all parts of the mass are subjected to high temperatures.

Foodborne disease caused by *Escherichia coli* O157:H7 has frequently been associated with contaminated ground beef, but illness caused by this pathogen is also now commonly linked to the consumption of a variety of fresh produce *(9, 13, 17, 24, 32, 52)*. Three large outbreaks of *E. coli* O157:H7 foodborne illness that occurred in 2006 were associated with leafy greens, and these events have further focused attention on livestock manures and composts as potential sources of pathogens for the contamination of these crops *(17, 60, 68)*. Pathogens in manure may directly contaminate food crops when the soil is amended with contaminated manure or compost *(20, 48),* or crop contamination may occur indirectly when soils, irrigation water, or floodwaters are contaminated by runoff from livestock production facilities, manure-amended fields, or stored manure before coming into contact with the crop *(21, 35)*. Treatment processes and management practices that effectively inactivate pathogens in livestock manures will reduce the risk of human foodborne illness linked to produce consumption.

Composting is a biological decomposition process used to manage organic waste materials such as manure, sewage sludge, household wastes, yard waste, and paper *(22, 55)*. Adequate mixtures of carbon and nitrogen (target C:N ratio of 20:1 to 40:1), water (moisture content of 40 to 65%), and oxygen (concentrations greater than 5%) promote microbial activity and the subsequent production of high temperatures that encourage the metabolic activity of thermophilic microorganisms in the organic materials *(22, 55)*. Periodic mixing of the organic materials maintains favorable composting conditions and promotes the continued generation of high temperatures in the compost piles. The heat generated by the thermophilic microbial activity is the primary factor that inactivates pathogens during compost-

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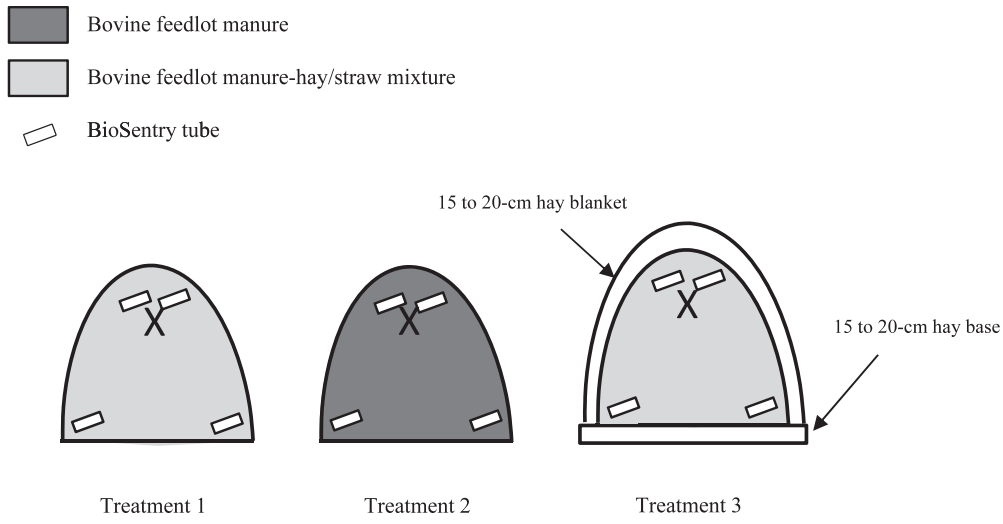

FIGURE 1. *Diagram of bovine feedlot surface manure composting treatments. Treatment 1 piles were a mixture of manure, hay, straw, and added water and were turned and mixed every 14 days. Treatment 2 piles were unamended stockpiled manure, which was piled and left static. Treatment 3 piles were a mixture of manure, hay, straw, and added water that was placed on a 15- to 20-cm-thick base of hay, covered with a 15- to 20-cm-thick blanket of hay, and left static. BioSentry tubes containing temperature data loggers and cassettes packed with manure were buried near the tops and toes of each pile as indicated in the diagram. At the top-center of the pile (X), the temperature was periodically measured with an analog compost thermometer. The initial piles were 1.5 to 1.8 m high and ca. 3.7 m in diameter at the base. In each of the two trials, four ca. 6.0-m^3 piles were constructed for each of the three composting treatments.*

ing, including both plant and zoonotic pathogens *(10, 34, 57)*. This pathogen reduction is an important benefit of the composting of livestock manure; additional benefits include the manufacture of a valuable product that is an excellent soil conditioner and has improved storage, handling, and land application qualities. Composting also reduces the nuisance odors and flies associated with manure.

Research with laboratory-scale composting bioreactors and/or inoculated bovine manure has revealed that properly controlled composting of bovine manure can inactivate *E. coli* O157:H7 *(34, 36, 45, 57)*. However, the inactivation of *E. coli* O157:H7 and other pathogens during on-farm or on-ranch composting of naturally contaminated bovine manure is likely far more variable. The nature of the farming enterprise in terms of the schedules and time required for crop and livestock management often reduces the time and attention available to actively manage composting of manure. Provision of the key conditions of balanced C:N, moisture, and aeration for effective composting requires effort on the part of the producer. Although on-farm composting of manure can typically be accomplished with existing farm equipment and facilities, actively managed composting costs time and money, in terms of labor, fuel, and special equipment *(55)*. As a result, many producers may resort to simple stockpiling of manure. With this approach, decomposition takes longer and may not achieve the same benefits for storage, handling, land application, fly reduction, and odor reduction as managed composting *(55)*. In addition, the inactivation of manure pathogens in stockpiled manure is likely incomplete and has not been fully examined. In the current study, we addressed these issues with an approach similar to that described by Arikan et al. *(1)*, and determined the abilities of on-farm minimally managed manure composting processes to inactivate naturally occurring *E. coli* O157:H7 in bovine feedlot surface manure. The impact of these processes on a selection of naturally present fecal bacterial populations and pathogens also was determined.

MATERIALS AND METHODS

Compost pile construction. This study was conducted at the U.S. Meat Animal Research Center (USMARC) near Clay Center, NE and consisted of two composting trials with identical treatments that were initiated in September 2007 (Trial 1) and in May 2008 (Trial 2). Before each trial, feedlot surface manure (FSM) from candidate pens at the USMARC feedlot (8 to 12 FSM samples of 300 to 400 g each from each pen) was screened to identify starting material with a high prevalence and/or levels of *E. coli* O157:H7 using procedures described below. For both trials, cattle were removed from the chosen pen, and the manure was scraped and brought to the composting site 1 to 2 days before the compost piles were constructed. Cattle in the selected pens were fed a ration composed of corn silage and alfalfa haylage and had been in these pens for 3 months (Trial 1) and 5 months (Trial 2) before the FSM was removed.

In each of the two trials, four ca. 6.0-m^3 compost piles of each of three composting treatment formats were constructed (Fig. 1; 12 piles for each trial). Treatment 1 compost piles were composed of a mixture of manure, hay, straw, and added water and were turned and mixed every 14 days up to day 56. In Trial 2, Treatment 1 piles were turned and mixed again a fifth time, on day 98. Treatment 2 compost piles were unamended stockpiled manure, which was piled and left static. Treatment 3 compost piles were constructed in a minimally managed composting format similar to a format described by Arikan et al. *(1)* and were composed of a mixture of manure, hay, straw, and added water that was placed on a 15- to 20-cm-thick base of hay, covered with a 15- to 20-cm-thick blanket of hay, and left static.

For those treatments composed of mixtures of manure, hay, straw, and added water, the materials were mixed on a concrete pad at the site with a front-end loader and a tractor and then divided

FIGURE 2. *BioSentry tube with cassettes containing manure.*

into separate piles. On a per-pile basis, these mixtures were composed of approximately 4.6 m^3 of manure, 1.5 m^3 of hay and straw, and 760 liters of water. A skid-steer with loader was used to form the manure and manure-hay-straw mixtures into conical piles 1.5 to 1.8 m in height and ca. 3.7 m in diameter at the base. Treatment 3 compost piles were loosely covered with a section of plastic deer netting, which was staked at the corners to keep the hay blankets in place. When Treatment 1 piles were turned and mixed, the materials from all Treatment 1 piles were removed to the concrete pad and mixed with the front-end loader and tractor and then redistributed and formed into the four compost piles at their original locations. For each trial, the compost pile treatments were randomized by block in a row running from east to west, with approximately 3.7 to 4.0 m between the piles (edge to edge).

Sample placement and collection. Subsamples of the unamended manure from the selected feedlot pens were packed into slotted tissue processing and embedding cassettes (Simport Macrosette, Beloeil, Quebec, Canada). Approximately 20 cassettes containing manure were then packed into BioSentry tubes *(1)* (Fig. 2) with one WatchDog 100 series button temperature logger (Spectrum Technologies, Plainfield, IL). The open slots of the cassettes, in addition to the holes along the length of the BioSentry tubes and the fiberglass screening on each end, allow the exchange of gas and heat between the bulk compost pile mass and the tube and cassette contents. Two filled BioSentry tubes were placed approximately 25 to 30 cm below the surface near the top of each compost pile, and two additional filled BioSentry tubes were placed 25 to 30 cm below the surface near the toe of each compost pile (Fig. 1). For one pile of each treatment, an additional duplicate filled BioSentry tube was placed in a toe position. BioSentry tubes were confirmed to be fully covered by and in contact with the bulk pile mass upon their initial placements and subsequent replacements in the compost piles.

Compost samples were collected on days 0, 3, 7, 14, 21, 28, 42, 56, and 84 of composting; samples in Trial 2 compost piles also were collected on day 126 of composting. On sampling days, the BioSentry tubes were recovered from the piles, cassettes were removed from each tube for microbial analyses, and then the tubes and their remaining contents were returned to their original locations in the piles. For those compost piles that were turned, the BioSentry tubes were removed and sampled and then returned to their original positions after the piles were mixed and reformed. For chemical analyses, ca. 500-g samples of the bulk pile material were collected near each tube location, and then pooled and mixed within pile and by location in the pile (top or toe). Immediately after collection, samples were transported to the laboratory for analyses.

Temperatures in the tops and toes of the piles were recorded hourly with the button temperature loggers. In addition, temperatures at three different locations slightly above the geometric center of each compost pile were measured periodically with an analog compost thermometer. To measure ambient air conditions, an additional BioSentry tube containing a WatchDog 102 series button temperature and relative humidity logger (Spectrum Technologies) was mounted 1.8 m above the ground and out of direct sunlight inside an open shed near the composting site.

Microbiological analyses. For the initial screening to identify manure for composting experiments, 10-g subsamples of each FSM sample collected from the feedlot pens was processed and analyzed to determine both the presence and level of *E. coli* O157:H7, using procedures previously described *(5, 7)*. Samples were weighed into separate sterile filtered sample bags (Nasco, Ft. Atkinson, WI), 90 ml of tryptic soy broth (TSB; BD, Franklin Lakes, NJ) was added, and the bag contents were mixed well by massaging the bag. For determination of *E. coli* O157:H7 levels, 1 ml was removed from the bag, and 50 μl was spiral plated with an Autoplate 4000 spiral plater (Spiral Biotech, Norwood, MA) onto CHROMagar O157 (DRG International, Mountainside, NJ) containing 5 mg/liter novobiocin and 2.5 mg/liter potassium tellurite (ntCHROM). The ntCHROM plates were incubated at 42°C for 18 to 20 h, and presumptive colonies were tested with *E. coli* O157 latex agglutination reagents (Oxoid, Basingstoke, UK). Agglutination-positive colonies were counted and confirmed by multiplex PCR for the *E. coli* genes *eaeA, slt-I, slt-II, fliC,* and *rfbE (33)*. The *fliC* primer sequences were those of Gannon et al. *(27),* and PCR conditions were those of Paton and Paton *(51)*. To determine the presence of *E. coli* O157:H7, the remaining initial 1:10 dilutions of FSM samples in TSB were incubated for 7 h at 37°C and then subjected to immunomagnetic separation (IMS). For IMS, 500 μl of each FSM sample was added to 500 μl of phosphate-buffered saline with Tween (PBS-Tween; Sigma, St. Louis, MO) and 20 μl of anti-O157 Dynabeads (Invitrogen, Carlsbad, CA). After 30 min of shaking at room temperature, the beads were removed from the sample, washed twice in 1 ml of PBS-Tween, and concentrated into 100 μl of PBS-Tween. Fifty microliters of each of the concentrated samples was spread plated onto an ntCHROM plate and a plate of sorbitol MacConkey agar (BD) containing 0.05 mg/liter cefixime and 2.5 mg/liter potassium tellurite (ctSMAC). Both ntCHROM and ctSMAC plates were incubated at 37°C for 22 to 24 h. Presumptive colonies were tested for agglutination and confirmed by multiplex PCR as described above.

For microbial analyses of compost samples from the piles, cassette contents (3 to 10 g) were emptied into separate sterile filtered sample bags and diluted 1:10 in TSB with a Smart Diluter (IUL Instruments, Neutec Group, Farmingdale, NY). The bag contents were mixed well by massaging. Subsamples of these initial dilutions were removed for the determination of bacterial populations and select pathogen analyses, and the remaining volumes were enriched at 37°C for 7 h before use in additional analyses for pathogens.

A portion of the initial 1:10 dilution in TSB was diluted as necessary in buffered peptone water, and suitable sample dilutions were spiral plated onto the appropriate agar medium. For determination of *E. coli* O157:H7 levels, samples were plated onto ntCHROM and incubated and analyzed as described above. Levels of *Clostridium difficile* spores in the initial 1:10 TSB

dilution were determined using procedures similar to those described by Borriello and Honour *(11)* with alcohol shock and plating onto prereduced *C. difficile* selective medium (CDMN) *(2),* which was incubated anaerobically at 37°C for up to 5 days. Presumptive colonies were examined as described below. For determination of total *E. coli* levels, samples were plated onto CHROMagar ECC (DRG International) and incubated at 37°C for 22 to 24 h; blue *E. coli* colonies were then counted. Levels of *Enterobacteriaceae* and aerobic bacteria were estimated with a Bactometer (bioMérieux, Hazelwood, MO), using the procedures described by Bosilevac et al. *(12).* General Purpose Medium Plus (bioMérieux) with added dextrose (18 g/liter) was used for the determination of aerobic bacteria, and Entero Medium (bioMérieux) was used for the determination of *Enterobacteriaceae*.

For Trial 1 only, levels of mesophilic and thermophilic bacteria were determined in compost samples collected throughout the composting period. The diluted samples described above were spiral plated onto tryptic soy agar (Difco, BD, Sparks, MD) containing 100 ppm of cycloheximide, and the plates were incubated for 48 h at 32°C and for 24 to 48 h at 55°C for determination of mesophilic bacteria and thermophilic bacteria, respectively.

To determine the presence of *Campylobacter* spp. in the compost samples, 1 ml of the initial 1:10 dilution in TSB was added to 13.5 ml of Bolton enrichment broth (BEB; Oxoid) in a sterile 15-ml conical tube and incubated at 37°C for 4 h and then at 42°C for 20 h. Twenty microliters of this BEB culture was then streaked onto Campy-Cefex plates *(64)* and incubated microaerobically at 42°C for 48 h in anaerobic jars containing CampyGen (Oxoid). Presumptive colonies were screened with Dryspot *Campylobacter* test agglutination reagents (Oxoid), and positive colonies were further confirmed and typed using the multiplex PCR primers and the procedure of Klena et al. *(40).*

The presence of *C. difficile* spores in the initial 1:10 TSB dilutions was determined using sample processing and enrichment procedures of Rodriguez-Palacios et al. *(54).* Colonies on CDMN agar plates that were positive for L-proline aminopeptidase (Pro Disc, Remel, Lenexa, KS) were subjected to PCR confirmation to identify the triose phosphate isomerase gene *(42).*

The presence of *E. coli* O157:H7 in the TSB enrichments of compost samples was determined by IMS, plating onto ntCHROM and ctSMAC, and multiplex PCR confirmation as described above for manure from the feedlot pens.

For determination of *Salmonella,* 1.0 ml of the primary TSB enrichments was further enriched in 9 ml of tetrathionate broth (BD) for 24 h at 37°C and then in Rappaport-Vassiliadis soya peptone (RVS) broth (Oxoid) for 16 to 18 h at 42°C. Twenty microliters of the enriched RVS broth was streaked onto a plate of brilliant green agar containing 80 mg/liter sodium sulfadiazine (BGS) and a plate of Hektoen enteric agar containing 15 mg/liter novobiocin *(3)* (both agars from BD), incubated for 24 h at 37°C, and examined for *Salmonella* colonies. The identities of suspect *Salmonella* colonies were confirmed by PCR for the *invA* gene as described by Berry and Siragusa *(4).*

A modification of the procedure of Guerini et al. *(29)* was used to determine the presence of *Listeria* spp. in compost samples. A 100-μl aliquot of the primary TSB enrichment was inoculated into Fraser broth (BD) and incubated at 35°C for 48 h. After this secondary enrichment, samples were plated onto *Listeria* CHROMagar (DRG International) and incubated at 37°C for 24 h. Blue colonies with or without haloes were confirmed as *Listeria* spp. or *Listeria monocytogenes* by multiplex PCR *(23).*

Chemical analyses. Moisture content of bulk pile compost samples (10 g) was determined by mass loss after drying overnight at 105°C. Organic matter content was determined as described by Miller and Berry *(47).* Sample pH (ION 2700 pH meter, Oakton, Vernon Hills, IL) and electrical conductivity (EC meter model 311, Corning, Corning, NY) were measured after mixing and shaking a 5-g compost sample in 25 ml of deionized water for 20 min. Total carbon and total nitrogen analyses (Leco combustion) were performed by a commercial laboratory (Ward Laboratories, Kearney, NE) *(46).*

Statistical analyses. Levels of total *E. coli, Enterobacteriaceae,* aerobic bacteria, heterotrophic bacteria, and thermophilic bacteria were transformed to log CFU per gram of FSM (wet weight) for statistical analyses. For samples in which *E. coli* levels were below the lower limit of detection of 200 CFU/g (2.3 log CFU/g), the value was set at one-half of the detection limit: 100 CFU/g (2.0 log CFU/g). The same approach was taken for samples in which aerobic bacteria and *Enterobacteriaceae* levels were below the lower limits of detection of 4.0 and 2.0 log CFU/g, respectively. The number of compost samples at each pile location (top or toe) of each treatment that were positive for *E. coli* O157:H7, *L. monocytogenes, Listeria* spp., and *Campylobacter* spp. at each sampling period were reported as a percentage. The experimental unit of observation was the compost pile. Least squares means of bacterial data were analyzed using the general linear models procedure (SAS Institute, Inc., Cary, NC). The analytical model included the effects of treatment, time, location in pile, treatment × time, location in pile × time, location in pile × treatment × time, and pile nested within treatment. Differences were considered significant when P values were less than 0.05, and were considered tendencies when P values were less than 0.10 but greater than 0.05.

RESULTS AND DISCUSSION

Many researchers have investigated manure composting in laboratory-scale composting bioreactors and have reported that the elevated temperatures typically generated during active composting are adequate to inactivate *E. coli* O157:H7 *(31, 36, 45).* Other researchers have verified these findings for *E. coli* O157:H7 during field-scale composting of livestock manures *(34, 57).* Laboratory strains of *E. coli* O157:H7 cultivated in artificial growth media, often labeled with convenient selection markers such as antibiotic resistance or green fluorescent protein, have been used for the inoculation of the manure in many of these studies *(36, 45, 57)* and may respond differently to composting than would naturally present or wild-type strains *(31).* In the present study, we sought to confirm the effectiveness of composting for reduction of naturally occurring *E. coli* O157:H7, present at levels more likely to be encountered in feedlot bovine manure. For Trial 1, all FSM samples in the selected pen were positive for *E. coli* O157:H7, and for five of the nine samples *E. coli* O157:H7 counts were 200 to 1,200 CFU/g. For Trial 2, 11 of the 12 FSM samples from the selected pen were positive for *E. coli* O157:H7, and all samples had levels lower than 200 CFU/g, which is the lower limit of detection of the enumeration procedure *(5).* Several researchers have reported levels of *E. coli* O157:H7 that may be shed by cattle. Typically, the majority of animals that are positive for this pathogen shed <200 CFU/g of feces; however, levels as high as 10^5 to 10^7 CFU/g have been reported *(14, 19, 50, 53, 69).* Recent studies have focused on the phenomenon of super shedder cattle, which

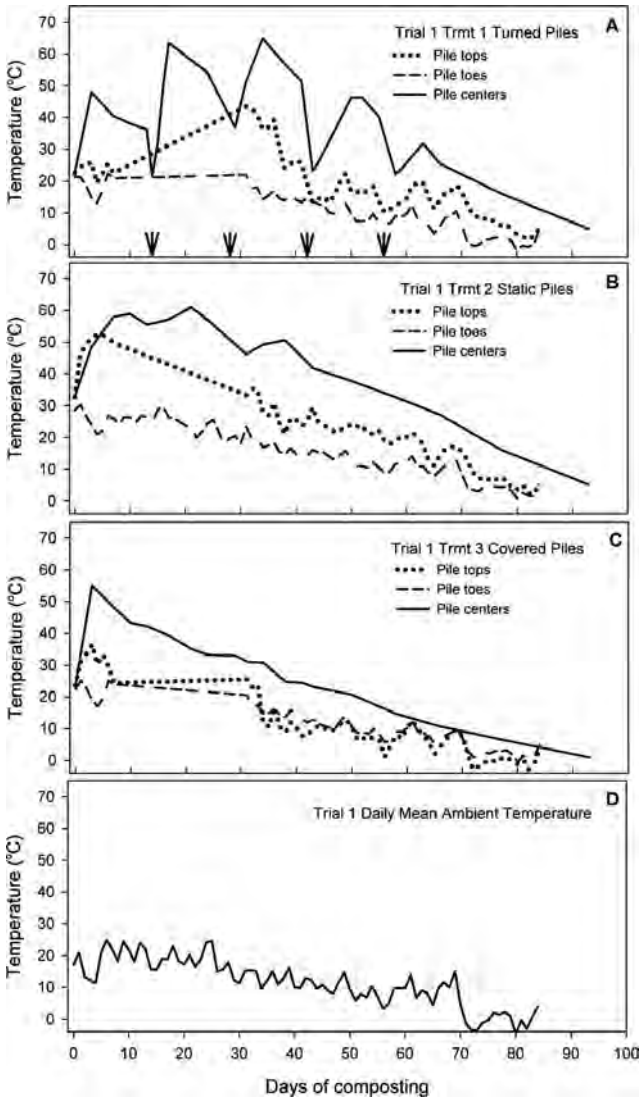

FIGURE 3. *Average temperature in the tops, toes, and centers of bovine feedlot manure compost piles constructed according to three different treatments, Trial 1. (A) Treatment 1, turned piles; (B) Treatment 2, static piles; (C) Treatment 3, covered piles; (D) daily mean ambient temperature. Treatment 1 piles (A) were turned on days 14, 28, 42, and 56 (arrows). Clock malfunctions in the temperature loggers during Trial 1 resulted in a 23-day gap (from day 8 through day 30) in temperature data for the tops and/or toes of some of the compost piles. For graphing and observation of trends, temperature data for these gaps was plotted as a straight line between days 7 and 31 (A through C).*

shed *E. coli* O157:H7 at $\geq 10^4$ CFU/g in their feces and can have a significant impact on the dissemination and maintenance of this pathogen in cattle production facilities *(18, 19)*. This research suggests that only a small proportion of cattle are super shedders, with reported percentages ranging from 0.45 to 9% of total animals *(19, 43, 50, 63)*. In comparison to information concerning fresh feces, information on typical levels of this pathogen that may occur in bovine manure accumulated on the feedlot pen surface is limited. With the continual mixing of feces, manure, and soil on the feedlot surface by hoof action and the likely gradual reduction of *E. coli* O157:H7 levels following fecal deposition, levels in FSM on the pen floor are anticipated to be lower than those found in the freshly voided feces. In previous work conducted during the summer months when the prevalence of *E. coli* O157:H7 typically is highest, we found that 12.8% of 250 FSM samples had *E. coli* O157:H7 levels of 200 CFU/g or higher, ranging from 200 CFU/g to 6.19 log CFU/g *(5)*. In a separate study that was conducted in July, 100% of FSM samples were positive for *E. coli* O157:H7, all at <200 CFU/g of FSM *(6)*. These findings suggest that the prevalence and levels of *E. coli* O157:H7 in the FSM used as the starting material for the compost piles likely is typical of those that one might expect to occur during periods of seasonally high *E. coli* O157:H7 prevalence, with the possible contribution of super shedding cattle, and thereby may represent a worst-case scenario with regard to naturally occurring *E. coli* O157:H7 in FSM destined for composting.

An additional goal of our work was to investigate minimally managed composting treatments and their ability to reduce *E. coli* O157:H7 and other manureborne bacteria. Treatment 1 compost piles most closely resembled actively managed piles *(66)*; in contrast, Treatment 2 compost piles were the least managed, being a passive composting process also referred to as aging or stockpiling *(22, 55, 67)*. The Treatment 3 compost pile configurations were similar to those of a minimally managed composting format evaluated by Arikan et al. *(1)*, who provided insulating layers of straw below and over the pile to reduce heat loss from the base and top. The compost samples for microbial analyses and the adjacent temperature loggers were positioned at the different locations in each pile to assess the anticipated differences in bacterial inactivation at pile locations predicted to heat to high temperatures (pile tops) or to remain at temperatures below those necessary to thoroughly destroy pathogens (pile toes). Periodic measurement with the compost thermometer of the temperature just above the geometric center of the piles (typically the hottest location in the pile, hereafter referred to as the pile center) was done to monitor the heating progress of the compost piles. To obtain adequate replication of results, we constructed four piles for each treatment in each of two trials.

Compost pile temperature profiles for Trials 1 and 2 are shown in Figures 3 and 4, respectively. Unfortunately, clock malfunctions in the temperature loggers during Trial 1 resulted in a 23-day gap (from day 8 through day 30) in temperature data for the tops and/or toes of some of the compost piles. For graphing and observation of trends, temperature data for these gaps were plotted as a straight line between days 7 and 31 (Figs. 3A through 3C). Generally, the temperatures in the center of the piles were hottest, followed by the pile tops, and the temperatures in the toes of the piles were coolest. We had anticipated that the temperatures in the pile tops would be more similar to those in the pile centers and hypothesize that heating and subsequent desiccation reduced the intimate contact between the temperature loggers and the pile mass, thereby affecting the temperature exposure of the loggers.

In Trial 1, the centers of the piles in all three composting treatments heated rapidly, reaching average temperatures above 45°C by day 3. Treatment 1 turned pile centers began to cool by day 7 but heated again to 64°C by

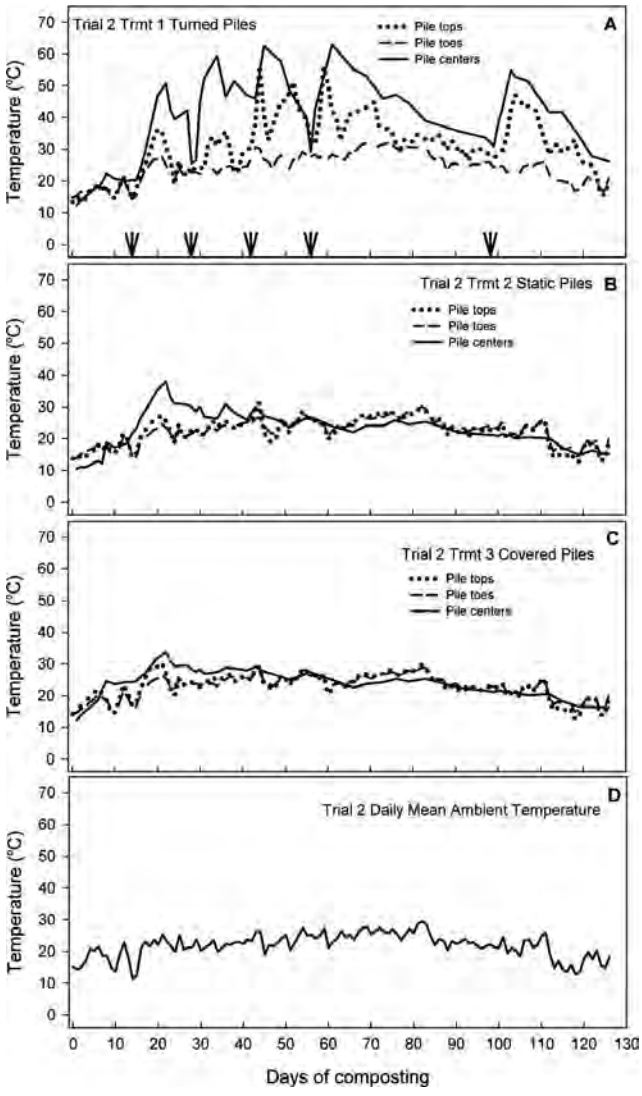

FIGURE 4. *Average temperature in the tops, toes, and centers of bovine feedlot manure compost piles constructed according to three different treatments, Trial 2. (A) Treatment 1, turned piles; (B) Treatment 2, static piles; (C) Treatment 3, covered piles; (D) daily mean ambient temperature. Treatment 1 piles (A) were turned on days 14, 28, 42, 56, and 98 (arrows).*

day 17, after the piles were turned on day 14 (Fig. 3A). This turning and heating pattern was repeated for each subsequent turning of the Treatment 1 piles on days 14, 28, and 42. Treatment 2 static pile centers were above 50°C for ca. 24 days and were above 40°C for ca. 42 days, and then cooled gradually throughout the remainder of the study period (Fig. 3B). Treatment 3 covered pile centers heated to 55°C by day 3 but thereafter gradually cooled and were below 40°C by day 17 (Fig. 3C). Little to no heating occurred in pile toes in all treatments, with temperatures remaining below 30°C and influenced primarily by the ambient temperature (Fig. 3D).

Comparison of the temperature profiles of all three composting treatments revealed that compost heating differed between Trials 1 and 2 (Figs. 3 and 4). Trial 2 Treatment 1 turned piles took longer to heat to high temperatures than did the piles of the same treatment in Trial 1 (Fig. 4A versus Fig. 3A). These piles did not heat substantially until after the piles were turned for the first time on day 14, reaching pile center temperature of 51°C by day 22. Thereafter, similar to the same pile treatment in Trial 1, the pile centers heated and then cooled after each turning, reaching temperatures of 59°C by day 34 and 63°C by day 45. In Trial 2, the length of the monitoring period was extended to 126 days. On day 98, Trial 2 Treatment 1 piles were turned and mixed again (a fifth time). Pile center temperatures heated to 55°C by day 103 and then cooled gradually to 26°C by day 126. Unlike the compost piles of the same treatments in Trial 1, the Treatment 2 and 3 piles in Trial 2 failed to adequately self-heat (Fig. 4B and 4C). For each treatment, pile center temperatures had reached their maximum by day 22, with Treatment 2 static piles reaching only 38°C and Treatment 3 covered piles reaching only 34°C. Through the remainder of Trial 2, temperatures in the centers, tops, and toes of piles of these two treatments were similar.

Current U.S. Department of Agriculture National Organic Program guidelines for compost use in organic crop production stipulate that compost piles must be turned or otherwise managed to ensure that all portions of the pile are heated to a minimum temperature of 55°C for at least 3 days *(65)*. By these standards, assuming that turning and mixing were adequate, only the Treatment 1 turned piles achieved this degree of heating. Although Trial 2 Treatment 2 static piles were above 55°C for more than 3 days in the centers, temperatures in the toes of these piles remained below 30°C.

Temperature profiles for the Treatment 1 turned piles of both trials are comparable to results reported for similarly managed bovine manure compost piles, with perhaps the exception of the delayed heating of the Trial 2 Treatment 1 compost piles *(41, 44, 56, 57)*. Likewise, the temperature profiles for the Trial 1 Treatment 2 static piles are comparable to reports describing the heating of stockpiled bovine manure compost piles that were left unturned *(1, 34, 44)*. However, our temperature observations for Treatment 3 covered piles are different from those in recent studies of the impact of coverings on composting of bovine manure. Insulating coverings have been used to retain heat within compost piles and to raise the temperature at peripheral locations of the pile to the higher temperatures needed for pathogen inactivation *(1, 56)*. Arikan et al. *(1)* placed amended bovine manure on a 6-inch (15.2-cm) layer of straw and covered the manure with another 6-inch layer of straw and found that the bottoms, middles, and tops of these static piles heated rapidly and remained above 50°C through 28 days of composting. Shepherd et al. *(56)* investigated the use of finished compost or straw on static piles of amended manure. With either covering, internal pile temperatures higher than 50°C were maintained for 8 to 12 days. However, with finished compost as a covering, temperatures at the interface of the manure and the covering were 7 to 15.5°C higher than that in straw-covered or uncovered piles. The 15- to 20-cm-thick blanket of hay that we employed to cover the Treatment 3 piles may have had greater porosity and/or provided insufficient insulation compared with the straw or finished compost used in these previous studies. The reasons for the difference in pile heating between our

TABLE 1. *Characteristics of feedlot surface manure (FSM) and FSM amended with hay and straw at day 0 and after 84 days of composting, Trials 1 and 2*[a]

Trial	Parameter	Day 0		Day 84 compost		
		FSM, Treatment 2	FSM + hay + straw, Treatments 1 and 3[b]	Treatment 1	Treatment 2	Treatment 3
Trial 1	Carbon content (%DM[c])	21.34 A	22.13 A	17.5 A	18.3 A	19.9 A
	Nitrogen content (%DM)	2.18 A	2.21 A	1.86 A	2.22 A	2.00 A
	C:N ratio	9.8:1 A	10:1 A	9.4:1 A	8.4:1 A	10:1 A
	pH	8.97 A	8.59 A	8.54 A	8.53 A	8.67 A
	Moisture content (%)	42.5 A	54.5 A	42.9 A	44.7 A	57.2 A
	Organic matter content (%)	45.3 A	45.0 A	35.3 A	38.4 A	39.2 A
	Electrical conductivity (mS/cm)	6.19 A	5.72 A	2.70 A	2.29 A	2.59 A
	Thermophilic bacteria (log CFU/g)	7.39 A	7.06 A	6.91 A	6.97 A	6.76 A
Trial 2	Carbon content (%DM)	15.6 B	17.5 B	11.6 B	13.5 B	14.4 B
	Nitrogen content (%DM)	2.99 B	3.14 B	2.07 A	2.34 A	2.24 A
	C:N ratio	5.2:1 B	5.6:1 B	5.6:1 B	6.0:1 B	6.7:1 B
	pH	8.81 A	8.63 A	8.42 A	8.34 A	8.30 A
	Moisture content (%)	55.3 B	53.9 A	43.1 A	35.5 A	31.8 B
	Organic matter content (%)	33.0 B	37.0 B	25.5 B	29.1 B	32.4 B
	Electrical conductivity (mS/cm)	2.71 B	2.66 B	2.20 B	2.90 B	3.10 B
	Thermophilic bacteria (log CFU/g)	6.64 B	6.96 A	7.12 A	6.15 B	6.08 A

[a] Values are means of determinations made with bulk samples collected from the tops and toes of each pile in each treatment group. Within day and treatment and across trials, means followed by the same letter are not significantly different ($P > 0.05$).
[b] Treatment 1 and 3 piles were composed of the same mixture of manure, hay, straw, and water.
[c] DM, dry matter.

Treatment 3 covered piles and the covered compost piles described by Arikan et al. *(1)* and Shepherd et al. *(56)* are uncertain but also may be associated with compositional differences in the initial amended and/or unamended manure feedstocks, such as C:N ratio, as discussed further below.

In keeping with our objective to investigate minimally managed composting systems, we did not analyze the composition or characteristics of our initial feedstock manure before pile construction. Although the FSM used in each trial was from cattle eating the same diet and the FSM mixtures with hay and straw were made in the same proportions, there were differences in biotic and abiotic characteristics of the initial FSM and amended FSM mixtures that may have played a role in the different compost pile heating results between Trials 1 and 2 (Table 1).

Composting guidelines suggest that the C:N ratio and moisture content of the feedstock materials be given primary consideration in developing mixtures for active composting *(22, 55)*. Recent work has demonstrated that both the initial C:N ratio and the moisture content of manure compost mixtures can influence the rate of pile heating and the rate of *E. coli* O157:H7 reduction *(59)*. The moisture content of the Trial 2 unamended FSM was 55.3%, and was higher ($P < 0.05$) than the 42.5% of the Trial 1 unamended FSM (Table 1). However, the initial moisture contents of the FSM and amended FSM of both Trials 1 and 2 were within the reasonable range for active composting *(22, 55)*. In contrast, the C:N ratios of the FSM and amended FSM for both Trials 1 and 2 were well below the optimum range. The recommended C:N ratio for rapid composting ranges from 20:1 to 40:1; mixtures with C:N ratios outside of this range can compost but the process may take longer or may be incomplete *(22, 55)*. In Trial 1, the unamended and amended FSM had C:N ratios of 9.8:1 and 10:1, respectively, but were significantly higher ($P < 0.05$) than the very low C:N ratios of the unamended and amended FSM used in Trial 2 compost, which were 5.2:1 and 5.6:1, respectively.

The organic matter contents of the initial unamended and amended FSM used in Trials 1 and 2 differed ($P < 0.05$). In Trial 1, the unamended and amended FSM had organic matter contents of 45.3 and 45.0%, respectively, which were significantly higher ($P < 0.05$) than those of the unamended and amended FSM used in Trial 2 compost, which were 33.0 and 37.0%, respectively (Table 1). The FSM used in Trials 1 and 2 had been accumulated in the feedlot pens for 3 and 5 months, respectively, and the organic matter analyses further indicate that the Trial 2 FSM contained a substantially higher proportion of aged decomposed manure than did the Trial 1 FSM, which likely played a role in constraining heat production during Trial 2 composting.

Electrical conductivity was significantly higher ($P < 0.05$) in Trial 1 unamended and amended FSM (6.19 and 5.72 mS/cm, respectively) than for Trial 2 unamended and amended FSM (2.71 and 2.66 mS/cm, respectively), but electrical conductivity of initial FSM from both trials were within ranges typical for bovine feedlot manure *(25)*.

The pH of the initial FSM and amended FSM were not different ($P > 0.05$) between Trials 1 and 2. These pHs ranged from 8.59 to 8.97 and were high but within the range of 5.5 to 9.0 that has been recommended for composting *(22, 55)*.

The eventual heating of the Trial 2 Treatment 1 turned piles is indicative of the forgiving nature of the composting process for inadequately balanced mixtures, given periodic

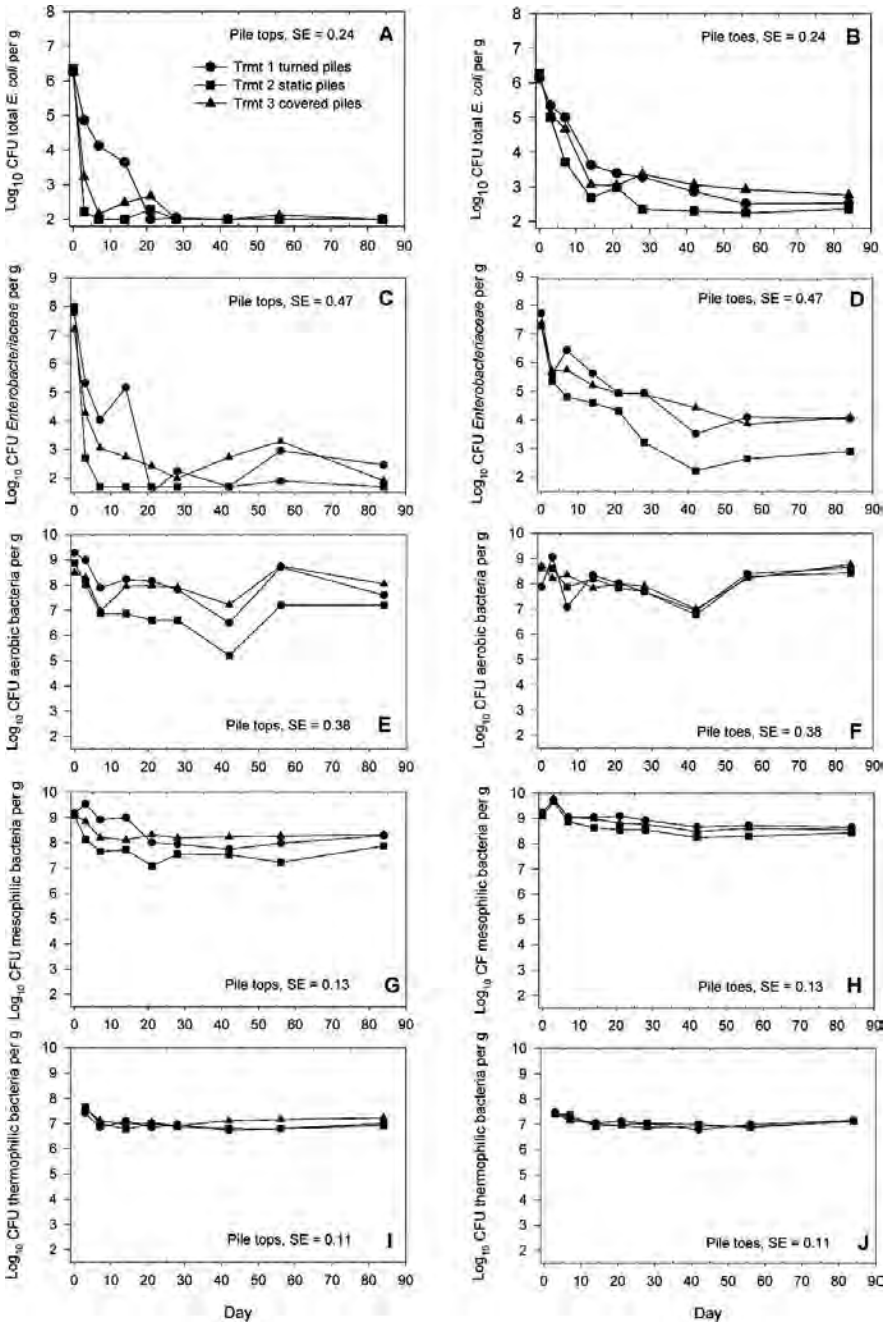

FIGURE 5. *Levels of total* E. coli *(A and B),* Enterobacteriaceae *(C and D), aerobic bacteria (E and F), mesophilic bacteria (G and H), and thermophilic bacteria (I and J) in samples from pile tops* (n = 8) *and toes* (n = 9) *during the composting of bovine feedlot manure in three different compost treatments, Trial 1. SE, standard error of the least squares means.*

turning and adequate time *(22)*; however, our results further highlight the importance of analyzing raw materials for preparing more effective composting mixtures, even for minimally managed composting schemes.

Successful aerobic composting relies upon the activity of both mesophilic and thermophilic microorganisms *(55).* We monitored the levels of mesophilic and thermophilic bacteria throughout the composting period in Trial 1 (Fig. 5G through 5J). Initial levels of mesophilic bacteria in all compost treatments ranged from 9.07 to 9.22 log CFU/g of compost (Fig. 5G and 5H) and were similar to or higher than levels reported in previous studies *(36, 56, 57).* After small increases for some treatments and pile locations, mesophilic bacteria decreased ($P < 0.05$) by 0.55 to 1.84 log CFU/g as composting progressed. However, mesophilic bacteria in all treatments remained higher than 7.00 log CFU/g in pile tops and higher than 8.20 log CFU/g in pile toes. In Trial 1, we also determined total aerobic bacteria in compost samples by impedance measured with a Bactometer. Average levels of total aerobic bacteria on day 0 for FSM and amended FSM were 7.88 to 9.27 log CFU/g (Fig. 5E and 5F), similar to the initial levels of mesophilic bacteria. Like the mesophilic bacteria, aerobic bacteria were not substantially reduced during the composting period. Thus, in Trial 2, total aerobic bacteria were enumerated by impedance in FSM and compost as a reasonable estimate of mesophilic bacteria levels. Aerobic bacteria levels in the initial FSM and amended FSM used in Trial 2 compost were similar to the levels observed in Trial 1 and ranged from 7.71 to 9.29 log CFU/g (Fig. 6E and 6F).

We were unable to obtain thermophilic bacteria counts for the Trial 1 day 0 compost because of inadequate sample

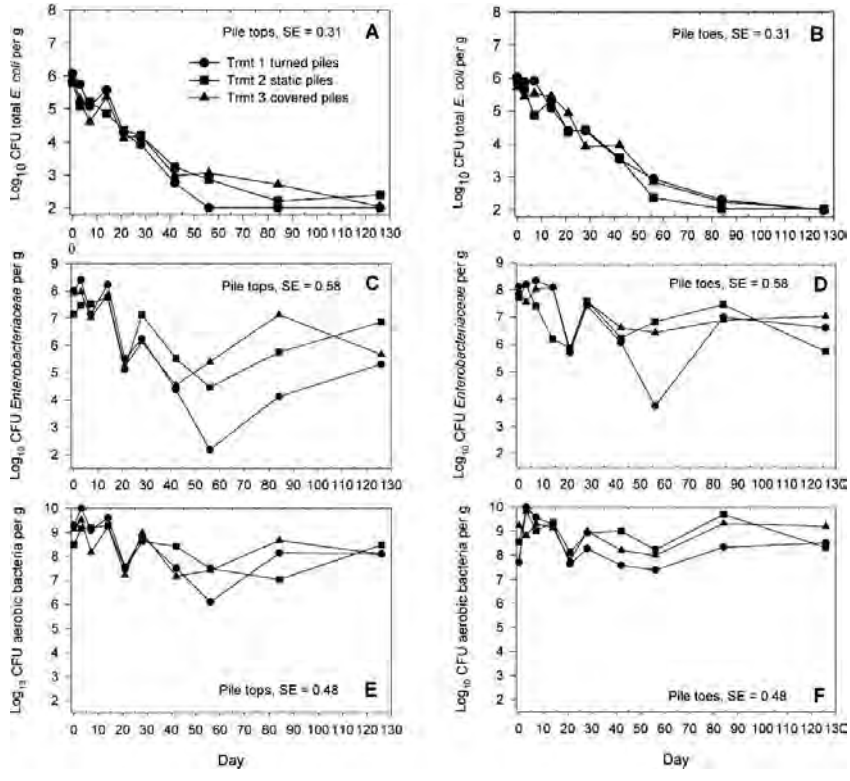

FIGURE 6. *Levels of total* E. coli *(A and B),* Enterobacteriaceae *(C and D), and aerobic bacteria (E and F) in samples from the pile tops (*n = *8) and pile toes (*n = *9) during the composting of bovine feedlot manure in three different compost treatments, Trial 2. SE, standard error of the least squares means.*

dilution. However, day 3 compost samples had thermophilic bacteria levels of 7.40 to 7.65 CFU/g of compost, similar to levels reported previously for initial bovine manure composting mixtures (Fig. 5I and 5J) *(56, 57).* After small decreases ($P < 0.05$) during the first 1 to 2 weeks of composting, thermophilic bacteria remained essentially unchanged ($P > 0.05$) through the remainder of the composting period for both the tops and toes of all treatment piles. We did not enumerate thermophilic bacteria in Trial 2 compost. Thus, to compare the initial levels of thermophilic bacteria in Trial 1 and 2 composts, retained frozen bulk samples of the starting FSM and unamended FSM were thawed, processed, and spiral plated as described above. Thermophilic bacteria levels were higher ($P < 0.05$) in initial FSM in Trial 1 than in Trial 2 (7.39 versus 6.64 log CFU/g) but were not different in the amended FSM (7.06 versus 6.96 log CFU/g) (Table 1). Although it is unclear whether the differences in thermophilic bacteria levels in the FSM were substantial enough to affect composting, these differences may explain in part the differences in pile heating that we observed between the two composting trials.

Seasonal and weather variations can affect the composting process *(22, 55)* and also may account for the difference in pile heating results between Trials 1 and 2. Composting for Trials 1 and 2 was conducted from September to December and May to September, respectively. Given the difference in thermophilic bacteria levels in the FSM used in the two trials (Table 1), accumulation of the FSM on the feedlot pen surface during the summer months may have selected for populations of thermophilic bacteria better suited for rapid composting in Trial 1. Feedlot pen surface temperatures as high as 50 to 55°C have been recorded at this site from May to September *(6, 15).* In experiments examining the composting of food waste in windrows during both winter and summer months, Cekmecelioglu et al. *(16)* observed seasonal differences in peak compost temperatures, duration of high compost temperatures, and inactivation of *Salmonella* and *E. coli* O157:H7, with faster reductions of the pathogens occurring during the summer trials. Shepherd et al. *(57)* observed higher survival of *E. coli* O157:H7 and total *E. coli* at the pile surface in bovine manure compost trials conducted in the fall compared with summer trials and concluded that this difference was due to the lower ambient temperatures. Seasonal differences in solar radiation or manure desiccation may also influence the survival of bacteria on the surfaces of compost piles *(34).*

In Trial 1, *E. coli* O157:H7 was present in 100% of day 0 samples for all compost pile treatments and locations (Fig. 7A and 7B). Among these samples, 39.2% had ≥200 CFU/g of compost (data not shown). The pathogen was sharply reduced in the pile top samples for all treatments during the first 7 days of composting ($P < 0.0001$) and was not recovered after 28 days of composting (Fig. 7A). For Treatment 2 static piles, 25% of top samples were positive for *E. coli* O157:H7 on day 3, but the pathogen was not recovered from top samples on day 7 or later. *E. coli* O157:H7 was isolated from 25% of top samples from Treatment 1 turned piles on day 14 but was not found thereafter. On day 21, 12.5% of top samples of Treatment 3 covered piles were positive for *E. coli* O157:H7, but all top samples were negative for the pathogen by day 28. *E. coli* O157:H7 was reduced in pile toe samples of all treatments ($P < 0.0001$) in spite of the lack of heating in these locations, although reduction of the pathogen was generally slower and/or incomplete compared with that in the pile tops (Fig. 7B). The pathogen was not recovered from toe

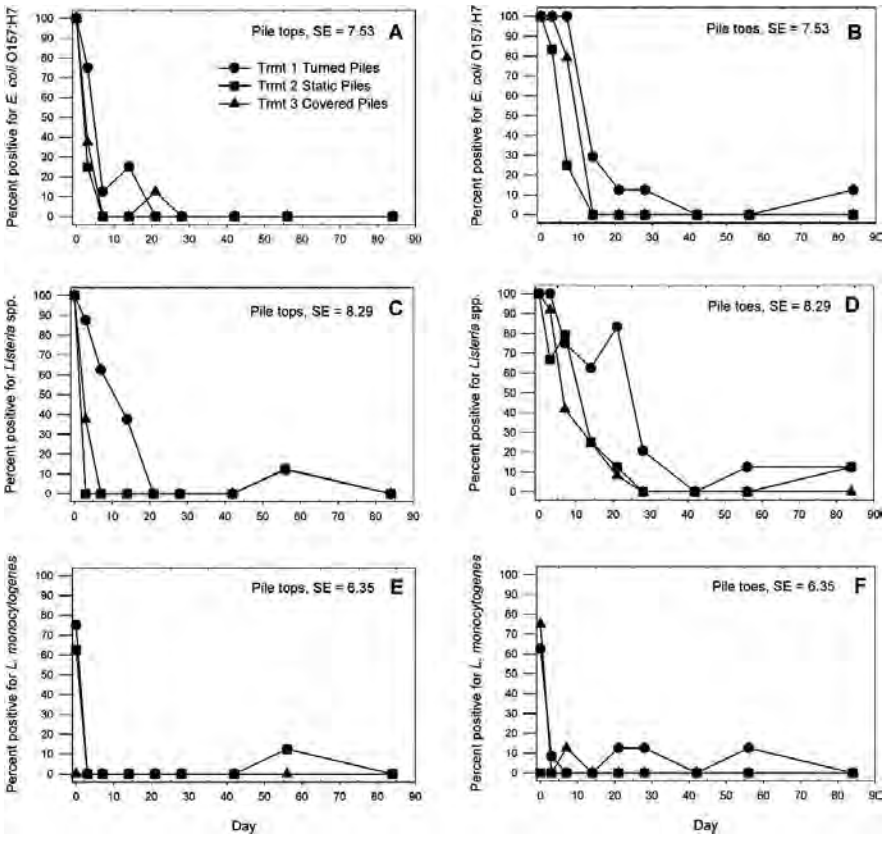

FIGURE 7. *Percentage of compost samples positive for* E. coli *O157:H7 (A and B),* Listeria *spp. (C and D), and* L. monocytogenes *(E and F) from the pile tops* (n = *8) and pile toes* (n = *9) during the composting of bovine feedlot manure in three different compost treatments, Trial 1. SE, standard error of the least squares means.*

samples of either Treatment 2 or Treatment 3 compost piles after 14 days of composting but was found in 12.5% of toe samples of Treatment 1 turned piles on days 21 and 28 and again on day 84 at the end of Trial 1.

Eighty-four percent of the initial compost samples for all treatments and locations in Trial 2 were positive for *E. coli* O157:H7 on day 0 (Fig. 8A and 8B). *E. coli* O157:H7 was reduced in Trial 2 compost piles of all treatments ($P < 0.0001$) despite the differences between the temperature profiles of the piles. However, the lack of heating in Treatment 2 and 3 compost piles may have extended the survival of the pathogen in the tops of the piles. *E. coli* O157:H7 was not isolated from the top of any pile in any treatment after 28 days of composting in Trial 1 but was recovered from Treatment 2 pile tops at day 42 and from Treatment 3 pile tops at day 56 in Trial 2. *E. coli* O157:H7 was not found in Trial 2 Treatment 1 turned pile toes at day 28 or later. Perhaps because of the similar temperatures of tops and toes in Treatment 2 piles, *E. coli* O157:H7 was recovered from Treatment 2 pile toe samples at day 42. In Trial 2, the pathogen was not isolated from pile toe samples of any treatment after 56 days.

Naturally occurring *E. coli* O157:H7 typically is present at much lower levels in bovine feedlot manure than in experimentally inoculated compost and at lower levels than naturally occurring appropriate indicator microorganisms *(5, 69)*. Thus, both total *E. coli* and *Enterobacteriaceae* were included in the microbial analyses of the compost as biological process indicators of *E. coli* O157:H7. Previous studies have revealed that the inactivation of total *E. coli* and *Enterobacteriaceae* during composting is correlated with the inactivation of *E. coli* O157:H7 *(56, 57)*. In Trial 1, the reduction of total *E. coli* in pile tops essentially mirrored that of *E. coli* O157:H7 in pile tops, for all treatments (Fig. 5A). By day 28 of composting, total *E. coli* was reduced ($P < 0.0001$) to below enumerable levels (threshold level: 2.3 log CFU/g of compost) in the pile tops of all treatments. However, total *E. coli* levels in pile toes of all treatments remained above detectable levels after 84 days of composting (Fig. 5B). In Trial 2 Treatment 1 turned piles, total *E. coli* levels were reduced ($P < 0.0001$) to below enumerable levels by day 56 in pile tops (Fig. 6A). Total *E. coli* levels also were reduced in the pile tops of those treatments that did not substantially heat in Trial 2; however, total *E. coli* remained at enumerable levels in Treatment 2 pile tops at day 126 and in Treatment 3 pile tops at day 84. The *E. coli* levels in the pile toe samples of all three treatments were below the detection limit at day 126 (Fig. 6B).

Initial levels of *Enterobacteriaceae* on day 0 were 7.15 to 8.12 log CFU/g of compost. In Trial 1, *Enterobacteriaceae* were reduced ($P < 0.0001$) in pile top samples by composting (Fig. 5C). In Treatment 2 static pile tops, average *Enterobacteriaceae* levels were reduced ($P < 0.0001$) to below the detection limit of 2.00 log CFU/g by day 7. In comparison, *Enterobacteriaceae* in tops of Treatment 1 turned piles and Treatment 3 covered piles was detectable at day 84. For all compost pile treatments in Trial 1, *Enterobacteriaceae* in pile toes were 2.88 log CFU/g or higher after 84 days of composting (Fig. 5D). In contrast to Trial 1, *Enterobacteriaceae* were not reduced below detectable levels in either pile tops or toes of any treatment

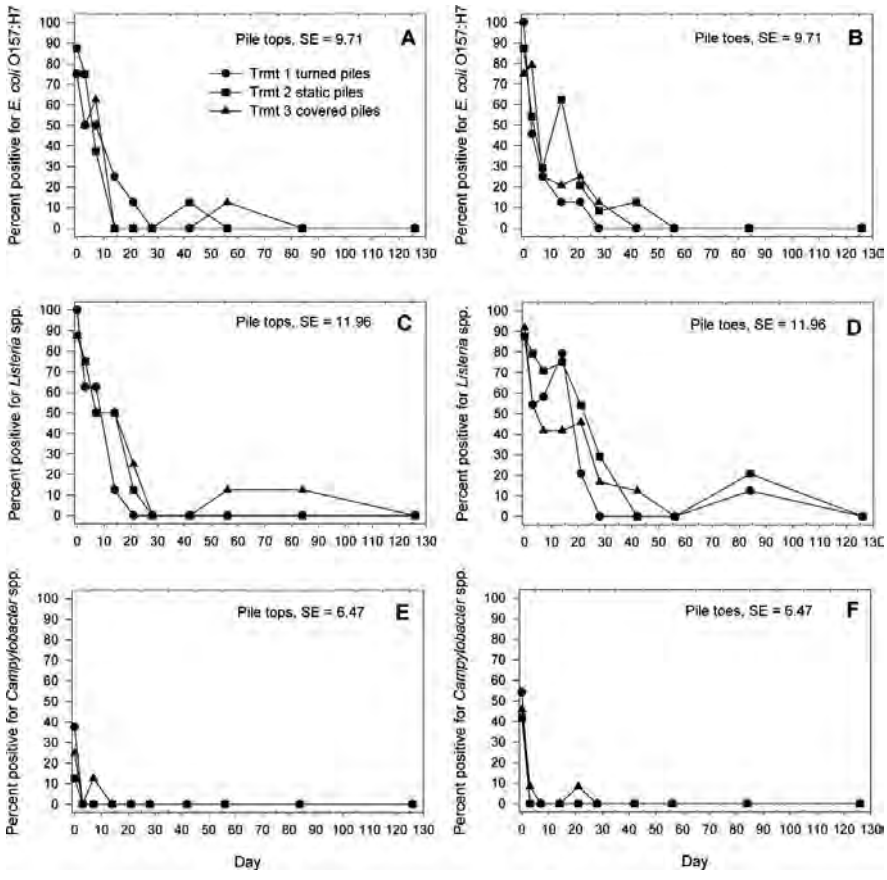

FIGURE 8. *Percentage of compost samples positive for* E. coli *O157:H7 (A and B),* Listeria *spp. (C and D), and* Campylobacter *spp. (E and F) from the pile tops (*n = *8) and pile toes (*n = *9) during the composting of bovine feedlot manure in three different compost treatments, Trial 2. SE, standard error of the least squares means.*

in Trial 2 (Fig. 6C and 6D). In the tops of the Treatment 1 turned piles, which did heat to high temperatures, *Enterobacteriaceae* were reduced ($P < 0.0001$) to an average of 2.18 log CFU/g by day 56; however, levels had increased ($P \leq 0.02$) to 4.13 and 5.31 log CFU/g by days 84 and 126, respectively. In the pile tops of Treatments 2 and 3, average *Enterobacteriaceae* levels were above 4.50 log CFU/g throughout the 126-day composting period. At 126 days, *Enterobacteriaceae* levels in the pile toe samples of all treatments in Trial 2 were 5.74 to 7.03 CFU/g. The general persistence of high levels of *Enterobacteriaceae,* in contrast to total *E. coli,* is likely a reflection of greater heterogeneity in heat resistance and survival characteristics of this broad group of bacteria in comparison to the single species *E. coli*.

Although the primary target of our work was *E. coli* O157:H7, we also determined the effects of composting on other zoonotic pathogens naturally present in FSM. *Salmonella* was detected only sporadically at the beginning and initial stages of composting in Trial 1 (data not shown) and was not detected in any samples during Trial 2. Although the prevalence of *Salmonella* was too low to fully assess the effects of the different compost treatments, the lack of *Salmonella*-positive samples during the later stages of composting in Trial 1 suggests that the treatments inactivated this pathogen from the initial levels. Several authors have described the effective reduction of *Salmonella* in manures by composting *(26, 34, 45, 49, 58).*

Listeria spp. were present in 100% of day 0 samples of all composting pile treatments in Trial 1 (Fig. 7C and 7D); in 48% of these samples, *L. monocytogenes* serovar 1/2b was isolated (Fig. 7E and 7F). *Listeria* spp. were not detected after enrichment from top samples of Treatments 1, 2, and 3 piles after 21, 3, and 7 days of composting, respectively, with the exception of the isolation of *Listeria* spp. at day 56 from 12.5% of pile top samples from each treatment (Fig. 7C). *Listeria* spp. persisted longer in pile toe samples in Trial 1 (Fig. 7D). *Listeria* spp. were not recovered from Treatment 3 covered pile toes after 28 days of composting but were found in 12.5% of pile toe samples of both Treatment 1 turned piles and Treatment 2 static piles at day 84. *L. monocytogenes* was not found in top samples of Treatment 3 piles during Trial 1 but was present at day 0 in 75.0 and 62.5% of Treatment 1 and Treatment 2 pile tops, respectively (Fig. 7E). *L. monocytogenes* was reduced ($P < 0.05$) from the tops of these treatment piles after 3 days of composting but was recovered from 12.5% of top samples on day 56. *L. monocytogenes* was not found in Trial 1 Treatment 2 toe samples but was present on day 0 in 62.5 and 75% of pile toe samples of Treatments 1 and 3, respectively (Fig. 7F). This organism was not found in Treatment 3 pile toe samples after 14 days of composting but was isolated up to day 56 in toe samples of Treatment 1 piles. *Listeria* spp. also were present in high percentages in the starting compost feedstock of Trial 2, at 87.5 to 100% in day 0 samples; however, only one isolate found in the top of a Treatment 1 turned pile on day 7 was confirmed as *L. monocytogenes* serovar 1/2b (data not shown). From initial percentages of 87.5 to 100%, *Listeria* spp. were reduced ($P < 0.05$) in pile top samples to below detectable levels by

21 days (Treatment 1) and 28 days (Treatment 2) of composting but were recovered from Treatment 3 pile tops after 56 and 84 days (Fig. 8C). *Listeria* spp. also were reduced in pile toe samples ($P < 0.05$) but were isolated from pile toe samples of all treatments at day 84 (Fig. 8D). In Trial 2, *Listeria* spp. were not recovered from either pile tops or toes of any composting treatment at day 126. Compared with *E. coli* O157:H7 and *Salmonella*, relatively fewer studies have examined the inactivation of *Listeria* spp. by composting of bovine manure *(34, 39, 49).* Similar to our observations, Hutchison et al. *(34)* found that 93 days of composting were required to reduce *L. monocytogenes* to levels below the detection limit in unturned piles of beef cattle manure. In contrast, Nicholson et al. *(49)* reported that *L. monocytogenes* inoculated at initial levels of 2.2 to 4.9 log CFU/g of manure survived a maximum of 4 days in turned and unturned piles of dairy cattle and swine manures.

No *Campylobacter* spp. were isolated from beginning manure, beginning manure mixtures with straw and hay, or compost during Trial 1. In Trial 2, *Campylobacter* spp. were present in 37.3% of the initial feedstock materials on day 0; 68% of these isolates were *Campylobacter jejuni* and 32% were *Campylobacter coli*. The FSM used in Trial 2 was accumulated in the feedlot pen from ca. December through May, and this seasonality of *Campylobacter* prevalence is consistent with our previous observation of high prevalence of this pathogen in runoff control system samples of this feedlot during the spring and winter months *(8).* On day 0, *Campylobacter* spp. were present in 37.5, 12.5, and 25.0% of pile top samples of Treatments 1, 2, and 3, respectively, and were not recovered from pile tops after 14 days of composting (Fig. 8E). Similarly, *Campylobacter* spp. were found in 54.3, 41.7, and 45.8% of pile toe samples of Treatments 1, 2, and 3 on day 0, respectively, and were not isolated from pile toes of any treatment after 28 days of composting (Fig. 8F). This reduction of *Campylobacter* spp. by composting is consistent with previous studies in which this pathogen in manure was inactivated by composting *(34, 39, 49).*

C. difficile was not recovered from initial manure or compost in either Trial 1 or Trial 2. Recent reports of the occurrence of this pathogen in retail meat products have led to the speculation of a foodborne route of transmission for *C. difficile (28, 54, 62).* This microorganism can cause disease in food animals, but more research is needed to clarify any connections between *C. difficile* in livestock and disease in humans *(28, 30, 61).* However, the common occurrence of *C. difficile* in food animals suggests that manure can serve as a source of this pathogen for food and water contamination. The thermal resistance of *C. difficile* spores may enhance its survival during manure composting, as has been observed for *Clostridium sporogenes (39).*

The recent foodborne pathogen outbreaks linked to produce have indicated the critical need for information that can be used to improve the safety of fruits and vegetables. Greater understanding of and improvements in the pathogen elimination steps in manure composting processes are needed to increase the safety of manure composts when they are used as soil amendments for high-risk fresh produce crops. This information is especially needed for organic produce crops for which manures and composts may be the only source of nutrients for soils used for their production. Our results suggest that given adequate time, minimally managed composting can reduce naturally occurring *E. coli* O157:H7 and other pathogens in bovine feedlot manure. However, the survival of both *Enterobacteriaceae* and total *E. coli* and the isolation of *E. coli* O157:H7 and *Listeria* spp. from toe samples of some compost piles in the later stages of composting point to the risks associated with incomplete inactivation of pathogens during composting. Shepherd et al. *(57)* found that *E. coli* O157:H7, total *E. coli,* and coliforms could survive up to 4 months on the surface of compost piles. Surviving *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* may regrow in bovine manure compost, further compounding the risk of pathogen contamination of food crops when this material is used for soil amendment or water contamination by runoff from amended soils or stored manure *(31, 37, 38).*

Our findings suggest specific management needs for the production of safe compost in low management scenarios. Among seasonal and compositional differences between the starting FSM feedstocks used in Trials 1 and 2, the low C:N ratio (5.2:1 to 5.6:1) in FSM and FSM mixtures in Trial 2 is most likely responsible for the lack of self-heating of Treatment 2 static piles and Treatment 3 covered piles in this trial. These observations support existing guidelines recommending C:N ratios of 20:1 to 40:1 in the starting materials to be composted *(22, 55)* and emphasize the results of other work demonstrating the importance of appropriate C:N formulations for rapid composting of bovine manure *(26, 59).* Our results also highlight the critical advantages of periodic turning of bovine manure compost piles. In spite of the low C:N ratio of the Trial 2 initial compost mixtures, Treatment 1 compost piles eventually heated to high temperatures after turning, whereas the static Treatment 2 and 3 piles did not. Furthermore, the persistence of *E. coli* O157:H7, *Listeria* spp., total *E. coli,* and *Enterobacteriaceae* in pile toe samples points to the importance of periodic turning of the piles to increase the likelihood that all parts of the mass are subjected to high temperatures for more effective and rapid elimination of pathogens.

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